WHOLE EXOME AND WHOLE GENOME SEQUENCING

Policy Number: LABORATORY 024.2 T2

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Related Policies

- Chromosome Microarray Testing
- Molecular Oncology Testing for Cancer Diagnosis, Prognosis, and Treatment Decisions

INSTRUCTIONS FOR USE

This Clinical Policy provides assistance in interpreting Oxford benefit plans. Unless otherwise stated, Oxford policies do not apply to Medicare Advantage members. Oxford reserves the right, in its sole discretion, to modify its policies as necessary. This Clinical Policy is provided for informational purposes. It does not constitute medical advice. The term Oxford includes Oxford Health Plans, LLC and all of its subsidiaries as appropriate for these policies.

When deciding coverage, the member specific benefit plan document must be referenced. The terms of the member specific benefit plan document [e.g., Certificate of Coverage (COC), Schedule of Benefits (SOB), and/or Summary Plan Description (SPD)] may differ greatly from the standard benefit plan upon which this Clinical Policy is based. In the event of a conflict, the member specific benefit plan document supersedes this Clinical Policy. All reviewers must first identify member eligibility, any federal or state regulatory requirements, and the member specific benefit plan coverage prior to use of this Clinical Policy. Other Policies may apply.

UnitedHealthcare may also use tools developed by third parties, such as the MCG™ Care Guidelines, to assist us in administering health benefits. The MCG™ Care Guidelines are intended to be used in connection with the independent professional medical judgment of a qualified health care provider and do not constitute the practice of medicine or medical advice.

CONDITIONS OF COVERAGE

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<tr>
<td>Authorization Required (Precertification always required for inpatient admission)</td>
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<tr>
<td>Precertification with Medical Director Review Required</td>
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<tr>
<td>Special Considerations</td>
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**BENEFIT CONSIDERATIONS**

Before using this policy, please check the member specific benefit plan document and any federal or state mandates, if applicable.

**Essential Health Benefits for Individual and Small Group**

For plan years beginning on or after January 1, 2014, the Affordable Care Act of 2010 (ACA) requires fully insured non-grandfathered individual and small group plans (inside and outside of Exchanges) to provide coverage for ten categories of Essential Health Benefits ("EHBs"). Large group plans (both self-funded and fully insured), and small group ASO plans, are not subject to the requirement to offer coverage for EHBs. However, if such plans choose to provide coverage for benefits which are deemed EHBs, the ACA requires all dollar limits on those benefits to be removed on all Grandfathered and Non-Grandfathered plans. The determination of which benefits constitute EHBs is made on a state by state basis. As such, when using this policy, it is important to refer to the member specific benefit plan document to determine benefit coverage.

**COVERAGE RATIONALE**

Genetic counseling is strongly recommended prior to these tests in order to inform persons being tested about the advantages and limitations of the test as applied to a unique person.

**Whole Exome Sequencing (WES)**

Whole Exome Sequencing (WES) is proven and/or medically necessary for diagnosing or evaluating a genetic disorder when the results are expected to directly influence medical management and clinical outcomes AND ALL of the following criteria are met:

- Clinical presentation is nonspecific and does not fit a well-defined syndrome for which a specific or targeted gene test is available. If a specific genetic syndrome is suspected, a single gene or targeted gene panel should be performed prior to determining if WES is necessary; and
- WES is ordered by a board-certified medical geneticist, neonatologist, neurologist, or developmental and behavioral pediatrician; and
- One of the following:
  - The clinical presentation or clinical and family history strongly suggest a genetic cause for which a specific clinical diagnosis cannot be made with any clinically available targeted genetic tests; or
  - There is a clinical diagnosis of a genetic condition where there is significant genetic heterogeneity and WES is a more practical approach to identifying the underlying genetic cause than are individual tests of multiple genes; or
  - There is likely a genetic disorder and multiple targeted gene tests that have failed to identify the underlying cause.

Comparator (e.g., parents or siblings) WES is proven and/or medically necessary for evaluating a genetic disorder when the above criteria have been met and WES is performed concurrently or has been previously performed on the individual.

WES is unproven and/or not medically necessary for all other indications, including but not limited to the following:

- Screening and evaluating disorders in individuals when the above criteria are not met
- Prenatal genetic diagnosis or screening
- Evaluation of fetal demise
- Preimplantation genetic diagnosis or screening in embryos
- Molecular profiling of tumors for the diagnosis, prognosis or management of cancer

Further studies are needed to evaluate the clinical utility of whole exome sequencing for other indications.

**Whole Genome Sequencing (WGS)**

Whole Genome Sequencing (WGS) is unproven and/or not medically necessary for screening and evaluating any disorder.

Although WGS has the potential to identify causal variants for a wide variety of conditions that may be missed with other technologies, as well as to identify predictive biomarkers, the information derived from WGS has not yet been translated into improved outcomes and changed medical management. Further studies are needed to establish the clinical utility of WGS.
DEFINITIONS

**Comparator**: A DNA sequence that is used to compare to the individual’s DNA sequence. This may be a parent or sibling of the individual, or non-cancerous tissue that is being compared to the individual’s tumor tissue (Thun et al., 2017).

**Next Generation Sequencing (NGS)**: New sequencing techniques that can quickly analyze multiple sections of DNA at the same time. Older forms of sequencing could only analyze one section of DNA at once.

**Variant of Unknown Significance (VUS)**: A variation in a genetic sequence that has an unknown association with disease. It may also be called an unclassified variant.

**Whole Exome Sequencing (WES)**: About 1% of a person’s DNA makes protein. These protein making sections are called exons. All the exons together are called the exome. WES is a DNA analysis technique that looks at all of the exons in a person at one time, rather than gene by gene (U.S. National Library of Medicine, 2017A).

**Whole Genome Sequencing (WGS)**: WGS determines the sequence of all of the DNA in a person, which includes the protein making (coding) as well as non-coding DNA elements (U.S. National Library of Medicine, 2017B).

APPLICABLE CODES

The following list(s) of procedure and/or diagnosis codes is provided for reference purposes only and may not be all inclusive. Listing of a code in this policy does not imply that the service described by the code is a covered or non-covered health service. Benefit coverage for health services is determined by the member specific benefit plan document and applicable laws that may require coverage for a specific service. The inclusion of a code does not imply any right to reimbursement or guarantee claim payment. Other Policies may apply.

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<td>Exome (e.g., unexplained constitutional or heritable disorder or syndrome); re-evaluation of previously obtained exome sequence (e.g., updated knowledge or unrelated condition/syndrome)</td>
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*CPT® is a registered trademark of the American Medical Association*

DESCRIPTION OF SERVICES

Whole Genome Sequencing (WGS) and Whole Exome Sequencing (WES) are increasingly clinically available due to significant advances in DNA sequencing technology over the last several years. (Taber et al., 2014) This testing approach, when applied to appropriate individuals and ordered and interpreted by medical specialists, can save both time and resources for individuals and their families (Beale et al., 2015; Frank et al., 2013; Canadian Agency for Drugs and Technologies in Health, 2014; American College of Medical Genetics and Genomics [ACMG], 2013). WES refers to the sequence determination of the exome. The exome is the portion of an individual’s genome that encodes protein (also known as exons). Approximately 1% of the genome is comprised of exons, which is about 30 million base pairs (Bertier, et al., 2016) and between 20,000-25,000 genes (U.S. National Library of Medicine, 2017C). Most known disease causing variants are found in the exons, and by sequencing them all simultaneously, a more efficient analysis can be completed than by sequencing each individual gene alone (Bertier et al., 2016). There is much that is unknown, however, and to date only 3,831 genes that are known to harbor one or more disease causing mutations have been identified (Online Mendelian Inheritance in Man, 2017).

WES results in long lists of genetic variants, and the success of this technology is dependent on how consistently and accurately labs can identify disease causing mutations (Richards et al., 2015).

The Clinical Sequencing Exploratory Research Consortium (CSER) studied variant assessment between nine labs performing exome analysis and applying the ACMG and AMP sequence variant interpretation guidelines, and found that intra-lab concordance was 79%, but inter-lab concordance was only 34%. After consensus efforts, 70% concordance was achieved between labs, reflecting the continued subjectivity. Five percent of the discordant interpretations would impact clinical care (Green et al., 2016).
In addition, because all genes are being analyzed simultaneously, an unexpected or incidental finding may be identified in the analysis that was outside of the clinical indication for the test (Richards et al., 2015). Novel variants may be discovered for the first time in the context of clinical care, laboratories that perform WES are in the unique position of requiring detailed clinical information to interpret results, and that may occasionally include testing of biological relatives (Richards et al., 2015).

WES may result in false positives due to the difficulty in reading CG rich regions, and poor coverage depth (Meienberg et al., 2016). A comparison of standard next generation sequencing (NGS) techniques and WES demonstrated that >98% of pathogenic variants are covered at depth adequate for detection (LaDuca et al., 2017).

For these reasons, it is critical that the ordering physician has specialty training and experience with these technologies and is prepared to work with the laboratory and interpret the results of such testing for their patient (Taber et al., 2014; Richards, et al., 2015; ACMG, 2012).

WGS refers to the sequence determination of most, but not all, of the DNA content comprising the entire genome of an individual representing about 3 billion base pairs, and covering all 20,000-25,000 genes and all exons (U.S. Library of Medicine, 2017A). As with WES, WGS results in long lists of unknown variants, and the methodology and databases available to interpret WGS are the same as WES, and focuses primarily on the exons (Richards et al., 2015; Landrum et al., 2015).

The functional implications of variants outside the exons are relatively unknown (Klein and Faroud, 2017). To date, only a small number of research articles have addressed the clinical utility of WGS. Recently several small studies have addressed the analytical validity of WGS as compared to WES, and found that WGS may provide more uniform coverage than WES, may more accurately detect a small number of variants compared to WES, and be better at identifying copy number variants (Belkadi et al., 2015; Meienberg et al., 2016). However, due to the data complexity, processing time, and interpretation time is much greater for WGS than for other NGS approaches (Klein and Faroud, 2017).

**CLINICAL EVIDENCE**

**Whole Exome Sequencing**

**WES Pediatric (Non-Cancer)**

Trujillano et al. (2017) reported on the results of WES performed on 1000 consecutive cases with suspected Mendelian disorders from 54 countries (78.5% Middle East, 10.6% Europe, and 10.9% from rest of the world) referred for diagnostic WES between January 2014 and January 2016. Patients ranged between 1 month and 59 years, 92.4% were 15 years or younger, with 14.1% younger than 1 year and 39.4% 1–5 years of age. The cohort also included 23 prenatal cases (2.3%). Notably, 45.3% of the cases were from consanguineous families and 38.1% presented family history of the disease. Most cases (82.7%) were analyzed with a trio design (parents and index). They identified pathogenic or likely pathogenic variants in 307 families (30.7%). In further 253 families (25.3%) a variant of unknown significance, possibly explaining the clinical symptoms of the index patient was identified. WES enabled timely diagnosing of genetic diseases, validation of causality of specific genetic disorders of PTPN23, KCTD3, SCN3A, PPOX, FRMPD4, and SCN1B, and setting dual diagnoses by detecting two causative variants in distinct genes in the same patient. There was a better diagnostic yield in consanguineous families, in severe and in syndromic phenotypes. Based on these results, the authors recommend WES as a first-line diagnostic in all cases without a clear differential diagnosis.

Tan et al. (2017) conducted a prospective analysis of the utility of WES on consecutive patients presenting at the Victorian Clinical Genetics Services at the Royal Children’s Hospital, Melbourne, Australia in 2015. These patients were older than 2 years of age and were suspected of having a monogenic disorder. The children had not previously had diagnostic testing, such as a single gene or gene panel test, but may have had a non-diagnostic microarray. All participants underwent WES with a phenotype driven data analysis. Of 61 children assessed, 44 underwent WES. A diagnosis was achieved in 23 by sequencing the child alone. The diagnosis was unanticipated in 8 children, and altered clinical management in 6. The range of ages was 2-18 years old. The average length of “diagnostic odyssey” was 6 years, and prior to WES the average number of clinical tests was 19, with 4 genetics consults and 4 consults with other specialists. Fifty nine children had undergone general anesthesia in order to perform a diagnostic test. The authors hypothesize that WES at the first indication of a genetic disorder would have reduced the number of tests and interventions, and provided an overall cost savings.

Vissers et al. (2017) of the Radboud University Medical Center in the Netherlands studied 150 consecutive patients presenting in the neurology clinic with non-acute neurological symptoms that were suspected to have a genetic origin, and compared the traditional work-up and testing paradigm to the use of WES. Both were conducted in parallel. The typical clinical approach gave a diagnostic yield of about 7%, whereas WES gave a diagnostic yield of about 29%. The authors highlighted the need for genetic counseling and tailored consent regarding incidental findings.
Tarallo-Graovac et al. (2016) combined deep clinical phenotyping (the comprehensive characterization of the discrete components of a patient's clinical and biochemical phenotype) with WES analysis through a semiautomated bioinformatics pipeline in consecutively enrolled patients with intellectual developmental disorder and unexplained metabolic phenotypes. WES was performed on samples obtained from 47 probands. Of these patients, 6 were excluded, including 1 who withdrew from the study. The remaining 41 probands had been born to predominantly nonconsanguineous parents of European descent. In 37 probands, the investigators identified variants in 2 genes newly implicated in disease, 9 candidate genes, 22 known genes with newly identified phenotypes, and 9 genes with expected phenotypes; in most of the genes, the variants were classified as either pathogenic or probably pathogenic. Complex phenotypes of patients in five families were explained by coexisting monogenic conditions. A diagnosis was obtained in 28 of 41 probands (68%) who were evaluated. A test of a targeted intervention was performed in 18 patients (44%). The authors concluded that deep phenotyping and WES in 41 probands with intellectual developmental disorder and unexplained metabolic abnormalities led to a diagnosis in 68%, the identification of 11 candidate genes newly implicated in neurometabolic disease, and a change in treatment beyond genetic counseling in 44%.

Stark et al. (2016) prospectively evaluated the diagnostic and clinical utility of singleton WES as a first-tier test in infants with suspected monogenic disease at a single pediatric tertiary center. This occurred in parallel with standard investigations, including single- or multigene panel sequencing when clinically indicated. The diagnosis rate, clinical utility, and impact on management of singleton WES were evaluated. Of 80 enrolled infants, 46 received a molecular genetic diagnosis through singleton WES (57.5%) compared with 11 (13.75%) who underwent standard investigations in the same patient group. Clinical management changed following exome diagnosis in 15 of 46 diagnosed participants (32.6%). Twelve relatives received a genetic diagnosis following cascade testing, and 28 couples were identified as being at high risk of recurrence in future pregnancies. The authors concluded that this prospective study provides strong evidence for increased diagnostic and clinical utility of singleton WES as a first-tier sequencing test for infants with a suspected monogenic disorder. Singleton WES outperformed standard care in terms of diagnosis rate and the benefits of a diagnosis, namely, impact on management of the child and clarification of reproductive risks for the extended family in a timely manner.

Retterer et al. (2015) reported the diagnostic yield of WES in 3,040 consecutive cases at a single clinical laboratory. WES was performed for many different clinical indications and included the proband plus two or more family members in 76% of cases. The overall diagnostic yield of WES was 28.8%. The diagnostic yield was 23.6% in proband-only cases and 31.0% when three family members were analyzed. The highest yield was for patients who had disorders involving hearing (55%, N = 11), vision (47%, N = 60), the skeletal muscle system (40%, N = 43), the skeletal system (39%, N = 54), multiple congenital anomalies (36%, N = 729), skin (32%, N = 31), the central nervous system (31%, N = 1,082), and the cardiovascular system (28%, N = 54). Of 2,091 cases in which secondary findings were analyzed for 56 American College of Medical Genetics and Genomics-recommended genes, 6.2% (N = 129) had reportable pathogenic variants. In addition to cases with a definitive diagnosis, in 24.2% of cases a candidate gene was reported that may later be reclassified as being associated with a definitive diagnosis. According to the authors, analysis of trios significantly improves the diagnostic yield compared with proband-only testing for genetically heterogeneous disorders and facilitates identification of novel candidate genes.

Valencia et al. (2015) performed a retrospective review of the first 40 clinical cases to determine the performance characteristics of WES in a pediatric setting by describing patient cohort, calculating the diagnostic yield, and detailing the patients for whom clinical management was altered. Of these, genetic defects were identified in 12 (30%) patients, of which 47% of the mutations were previously unreported in the literature. Among the 12 patients with positive findings, seven had autosomal dominant disease and five had autosomal recessive disease. Ninety percent of the cohort opted to receive secondary findings and of those, secondary medical actionable results were returned in the literature. Among the 12 patients with positive findings, seven had autosomal dominant disease and five had autosomal recessive disease. Ninety percent of the cohort opted to receive secondary findings and of those, secondary medical actionable results were returned in three cases. The diagnostic workup included a significant number of genetic tests with microarray and single-gene sequencing being the most popular tests. Genetic diagnosis from WES led to altered patient medical management in positive cases. The authors concluded that this review demonstrates the clinical utility of WES by establishing the clinical diagnostic rate and its impact on medical management in a large pediatric center. The cost-effectiveness of WES was demonstrated by ending the diagnostic odyssey in positive cases. According to the authors, in some cases it may be most cost-effective to directly perform WES.

Farwell et al. (2015) provided the results from the first 500 probands referred to a clinical laboratory for diagnostic exome sequencing. Family-based exome sequencing included WES followed by family inheritance-based model filtering, comprehensive medical review, familial cosegregation analysis, and analysis of novel genes. A positive or likely positive result in a characterized gene was identified in 30% of patients (152/500). A novel gene finding was identified in 7.5% of patients (31/416). The highest diagnostic rates were observed among patients with ataxia, multiple congenital anomalies, and epilepsy (44, 36, and 35%, respectively). Twenty-three percent of positive findings were within genes characterized within the past 2 years. The diagnostic rate was significantly higher among families undergoing a trio (37%) as compared with a singleton (21%) whole-exome testing strategy. According to the authors,
data demonstrate the utility of family-based exome sequencing and analysis to obtain the highest reported detection rate in an unselected clinical cohort, illustrating the utility of diagnostic exome sequencing as a transformative technology for the molecular diagnosis of genetic disease.

Yang et al. (2014) performed clinical whole-exome sequencing and reported (1) the rate of molecular diagnosis among phenotypic groups, (2) the spectrum of genetic alterations contributing to disease, and (3) the prevalence of medically actionable incidental findings such as FBN1 mutations causing Marfan syndrome. This was an observational study of 2000 consecutive patients with clinical WES analyzed between June 2012 and August 2014. WES tests were performed at a clinical genetics laboratory in the United States. Results were reported by clinical molecular geneticists certified by the American Board of Medical Genetics and Genomics. Tests were ordered by the patient’s physician. The patients were primarily pediatric (1756 [88%]; mean age, 6 years; 888 females [44%], 1101 males [55%], and 11 fetuses [1% gender unknown]), demonstrating diverse clinical manifestations most often including nervous system dysfunction such as developmental delay. A molecular diagnosis was reported for 504 patients (25.2%) with 58% of the diagnostic mutations not previously reported. Molecular diagnosis rates for each phenotypic category were 143/526 for the neurological group, 282/1147 for the neurological plus other organ systems group, 30/83 for the specific neurological group, and 49/244 for the non-neurological group. The Mendelian disease patterns of the 527 molecular diagnoses included 280 (53.1%) autosomal dominant, 181 (34.3%) autosomal recessive (including 5 with uniparental disomy), 65 (12.3%) X-linked, and 1 (0.2%) mitochondrial. Of 504 patients with a molecular diagnosis, 23 (4.6%) had blended phenotypes resulting from 2 single gene defects. About 30% of the positive cases harbored mutations in disease genes reported since 2011. There were 95 medically actionable incidental findings in genes unrelated to the phenotype but with immediate implications for management in 92 patients (4.6%), including 59 patients (3%) with mutations in genes recommended for reporting by the American College of Medical Genetics and Genomics. The authors concluded that WES provided a potential molecular diagnosis for 25% of a large cohort of patients referred for evaluation of suspected genetic conditions, including detection of rare genetic events and new mutations contributing to disease. According to the authors, the yield of WES may offer advantages over traditional molecular diagnostic approaches in certain patients.

Lee et al. (2014) reported on initial clinical indications for clinical exome sequencing (CES) referrals and molecular diagnostic rates for different indications and for different test types. Clinical exome sequencing was performed on 814 consecutive patients with undiagnosed, suspected genetic conditions between January 2012 and August 2014. Clinical exome sequencing was conducted as trio-CES (both parents and their affected child sequenced simultaneously) to effectively detect de novo and compound heterozygous variants or as proband-CES (only the affected individual sequenced) when parental samples were not available. Of the 814 cases, the overall molecular diagnosis rate was 26%. The molecular diagnosis rate for trio-CES was 31% and 22% for proband-CES. In cases of developmental delay in children (<5 years, n = 138), the molecular diagnosis rate was 41% for trio-CES cases and 9% for proband-CES cases. The significantly higher diagnostic yield of trio-CES was due to the identification of de novo and compound heterozygous variants. The authors concluded that in this sample of patients with undiagnosed, suspected genetic conditions, trio-CES was associated with higher molecular diagnostic yield than proband-CES or traditional molecular diagnostic methods.

Several other studies that evaluated WES have also reported that the diagnosis directly changed patient management (Soden et al., 2014; Srivastava et al., 2014; Zhu et al., 2015; Nolan and Carlson, 2016). WES results changed patient management in 45.3% to 76.9% of patients in 3 studies and 3.4% in 1 study. Changes to patient management included changes to drug or diet and referrals to other physicians/monitoring; results were reported to also change other family member’s family planning and to guide their genetic testing. One study (Nolan and Carlson, 2016) reported on incidental findings (i.e., findings with clinical significance, such as a variant with a known disease-causing effect, but for a different condition than that being studied), which resulted in screening or monitoring of the patient (Hayes, 2016).

**WES Prenatal**

There are limited data on WES in prenatal genetic diagnostic testing. Fu et al. (2017) did sequential analysis involving karyotype, chromosome microarray (CMA), and then WES in a cohort of 3949 structurally abnormal fetuses. Eighteen percent (720) fetuses had an abnormal karyotype. CMA analysis was performed on those with a normal karyotype (1680) and 8% (168) had a pathogenic copy number variant. Of those with a normal karyotype and CMA analysis, 196 underwent WES, and 47 (24%) had a pathogenic variant identified that could potentially explain the phenotype; additionally, the incidence of variants of unknown significance (VUS) and secondary findings was 12% and 6%, respectively. Further studies are needed to establish the clinical validity and clinical utility of WES in this setting.

**WES Adult (Non-Cancer)**

Posey et al. (2016) performed a retrospective analysis of consecutive WES reports for adults from a diagnostic laboratory. Phenotype composition was determined using Human Phenotype Ontology terms. Molecular diagnoses were reported for 17.5% (85/486) of adults, lower than a primarily pediatric population (25.2%; p=0.0003); the diagnostic rate was higher (23.9%) in those 18–30 years of age compared to patients over 30 years (10.4%;
p=0.0001). Dual Mendelian diagnoses contributed to 7% of diagnoses, revealing blended phenotypes. Diagnoses were more frequent among individuals with abnormalities of the nervous system, skeletal system, head/neck, and growth. Diagnostic rate was independent of family history information, and de novo mutations contributed to 61.4% of autosomal dominant diagnoses. This early WES experience in adults demonstrates molecular diagnoses in a substantial proportion of patients, informing clinical management, recurrence risk and recommendations for relatives. A positive family history was not predictive, consistent with molecular diagnoses often revealed by de novo events, informing the Mendelian basis of genetic disease in adults. Additional studies in WES sequencing are needed to validate its clinical utility.

**WES Cancer**

Patients with metastatic and treatment-resistant cancer were prospectively enrolled at a single academic center for paired metastatic tumor and normal tissue WES during a 19-month period. (Beltran et al., 2015) A comprehensive computational pipeline was used to detect point mutations, indels, and copy number alterations. Mutations were categorized as category 1, 2, or 3 on the basis of level of potential action; clinical reports were generated and discussed in precision tumor board. Patients (n=97, with 154 tumor pairs) were observed for 7 to 25 months for correlation of molecular information with clinical response. Results showed that more than 90% of patients harbored actionable or biologically informative alterations, although treatment was guided by the information in only 5% of cases. This study highlights opportunities for future clinical trials regarding whole-exome sequencing in precision medicine.

Malhotra et al. (2014) evaluated whether there is evidence that WES improves outcomes for patients with cancer. Published evidence was evaluated using a methodology that combines the analytical validity, clinical validity, clinical utility and ethical, legal, and social implications (ACCE) model for genetic test evaluations with internationally accepted health technology assessment methodology. WES has been conducted most extensively (seven studies to date) in breast cancer patients, with fewer studies of other types of cancers (e.g., leukemia, prostate cancer, and ovarian cancer). Studies evaluating somatic alterations showed high intratumor and inter-tumor heterogeneity. In addition, both novel and previously implicated variants were identified. However, only three studies have shown potential for clinical utility of WES; whereby, variants identified through WES may determine response to drug treatment. The authors concluded that despite evidence for clinical validity of WES in cancers, clinical utility is very limited and needs to be further evaluated in large clinical studies.

In a breast cancer study, follow-up analyses showed enrichment of GATA3 variants (identified by WES) in samples showing a decline in Ki-67 levels, which is a marker for response to aromatase inhibitor treatment. This association suggests that presence of GATA3 variants may be a predictive marker to identify individuals who will respond to treatment with aromatase inhibitors (Ellis et al., 2012). A second study showed that somatic hypervariation detected through WES not only was a predictive factor for determining platinum-based chemotherapy response in ovarian cancer treatment, but was also statistically significantly associated with longer overall survival and progression-free survival (Sohn et al., 2012). Another WES study by Tzoneva et al. (2013) identified NT5C2 variants that were associated with AML relapse even when receiving treatment, and early relapse compared to late relapse, suggesting NT5C2 may be a potential marker to identify individuals who may experience AML relapse despite chemotherapy treatment. While these 3 studies suggest potential for clinical utility, they need to be validated in larger clinical studies.

**Whole Genome Sequencing**

**WGS Pediatric (Non-Cancer)**

Bowling et al. (2017) report results of WES or WGS on 371 individuals with developmental delay or intellectual disabilities enrolled in the Clinical Sequencing Exploratory Research (CSER) consortium (WES for 127 and WGS for 244) A total of 284 participating families were enrolled with both biological parents and 35 affected individuals had one parent included. Mean age of study participants was 11 years and 58% were male. Affected individuals displayed symptoms described by 333 unique Human Phenotype Ontology terms with over 90% of individuals displaying intellectual disability, 69% with speech delay, 45% with seizures, and 20% with microcephaly or macrocephaly; 18% had an abnormal brain magnetic resonance imaging (MRI) result and 81% had been subjected to prior genetic testing. Pathogenic or likely pathogenic variants were found in 100 individuals (27%), with variants of uncertain significance in an additional 42 (11%). The pathogenic or likely pathogenic identification rate was not significantly different between WES or WGS (p = 0.30) for single nucleotide variants or small insertions or deletions; although WGS can also identify copy number variants.

In a prospective study, Stavropoulos et al. (2016) utilized WGS and comprehensive medical annotation (CMA) to assess 100 patients referred to a pediatric genetics service, and compared the diagnostic yield versus standard genetic testing. WGS identified genetic variants meeting clinical diagnostic criteria in 34% of cases, representing a fourfold increase in diagnostic rate over CMA alone and more than twofold increase in CMA plus targeted gene sequencing. WGS identified all rare clinically significant CNVs that were detected by CMA. In 26 patients, WGS revealed indel and missense mutations presenting in a dominant (63%) or a recessive (37%) manner. The
investigators found four subjects with mutations in at least two genes associated with distinct genetic disorders, including two cases harboring a pathogenic CNV and SNV. In the authors’ opinion, when considering medically actionable secondary findings in addition to primary WGS findings, 38% of patients would benefit from genetic counselling. While promising, additional studies of WGS as a primary test in comparison to conventional genetic testing and WES are needed.

Bodian et al. (2016) assessed the potential of WGS to replicate and augment results from conventional blood-based newborn screening (NBS). Research-generated WGS data from an ancestrally diverse cohort of 1,696 infants and both parents of each infant were analyzed for variants in 163 genes involved in disorders included or under discussion for inclusion in US NBS programs. WGS results were compared with results from state NBS and related follow-up testing. NBS genes are generally well covered by WGS. There is a median of one (range: 0-6) database-annotated pathogenic variant in the NBS genes per infant. Results of WGS and NBS in detecting 28 state-screened disorders and four hemoglobin traits were concordant for 88.6% of true positives (n = 35) and 98.9% of true negatives (n = 45,757). Of the five infants affected with a state-screened disorder, WGS identified two whereas NBS detected four. WGS yielded fewer false positives than NBS (0.037 vs. 0.17%) but more results of uncertain significance (0.90 vs. 0.013%). The authors concluded that WGS may help rule in and rule out NBS disorders, pinpoint molecular diagnoses, and detect conditions not amenable to current NBS assays. There is a need for additional studies that compare WGS with traditional NBS methods and evaluate the change in patient management resulting from WGS for newborn screening.

Taylor et al. (2015) conducted a study to assess factors influencing the success of WGS to obtain a genetic diagnosis across a broad range of clinical conditions with no previously identified causal mutation. They sequenced 217 individuals from 156 independent cases or families across a broad spectrum of disorders in which previous screening had identified no pathogenic variants. The investigators quantified the number of candidate variants identified using different strategies for variant calling, filtering, annotation and prioritization. They found that jointly calling variants across samples, filtering against both local and external databases, deploying multiple annotation tools and using familial transmission above biological plausibility contributed to accuracy. Overall, the investigators identified disease-causing variants in 21% of cases, with the proportion increasing to 34% (23/68) for Mendelian disorders and 57% (8/14) in family trios. They also discovered 32 potentially clinically actionable variants in 18 genes unrelated to the referral disorder, although only 4 were ultimately considered reportable. According to the investigators, their results demonstrate the value of genome sequencing for but also highlight many outstanding challenges, including the challenges of interpreting unrelated variants.

Willig et al. (2015) performed a retrospective comparison of rapid whole-genome sequencing (STATseq) and standard genetic testing in a case series from the neonatal and pediatric intensive care units (NICU and PICU) of a large children's hospital. The participants were families with an infant younger than 4 months with an acute illness of suspected genetic cause. The intervention was STATseq of trios (both parents and their affected infant). The main measures were the diagnostic rate, time to diagnosis, and rate of change in management after standard genetic testing and STATseq. Twenty (57%) of 35 infants were diagnosed with a genetic disease by use of STATseq and three (9%) of 32 by use of standard genetic testing. Median time to genome analysis was 5 days (range 3-153) and median time to STATseq report was 23 days. Thirteen (65%) of 20 STATseq diagnoses were associated with de-novo mutations. Impact on clinical management was noted in 13 (65%) of 20 infants with a STATseq diagnosis, four (20%) had diagnoses that led to a clinical intervention and six (30%) were started on palliative care. The 120-day mortality was 57% (12 of 21) in infants with a genetic diagnosis. According to the authors, in selected acutely ill infants, STATseq had a high rate of diagnosis of genetic disorders. The authors indicated that while having a genetic diagnosis altered the management of infants in the NICU or PICU in this single institution; additional studies with a higher patient population are needed to validate the clinical utility of WGS in this patient population.

Soden et al. (2014) reported on one hundred families with 119 children affected by neurodevelopmental disorders (NDD) who had WGS, WES, or WES followed by WGS of parent-child trios, with the sequencing approach guided by acuity of illness. Forty-five percent received molecular diagnoses. An accelerated sequencing modality, rapid WGS, yielded diagnoses in 73% of families with acutely ill children (11 of 15). Forty percent of families with children with nonacute NDD, followed in ambulatory care clinics (34 of 85), received diagnoses: 33 by WES and 1 by staged WES then WGS. A change in clinical care or impression of the pathophysiology was reported in 49% of newly diagnosed families. According to the authors, if WES or WGS had been performed at symptom onset, genomic diagnoses may have been made 77 months earlier. It is suggested that initial diagnostic evaluation of children with NDD should include trio WGS or WES, with extension of accelerated sequencing modalities to high-acuity patients. According to the authors, this study had several limitations. It was retrospective and lacked a control group. Clinical data were collected principally through chart review, which may have led to underestimate or overestimates of changes in clinical management. The authors did not ascertain information about long-term consequences of diagnosis, such as the impact of genetic counseling. Comparisons of costs of genomic and conventional diagnostic testing excluded associated costs of testing, such as outpatient visits, and may have included tests that would nevertheless have been performed, irrespective of diagnosis. The acuity-based approach to expedited WGS and non-expedited WES was a patient care-driven approach and was not designed to facilitate direct comparisons between the two methods.
WGS Other (Non-Cancer)

Ellingford et al. (2016) compared the use of next generation gene targeted next generation sequencing (NGS) with WGS in a nested cohort of 46 (of 562) people with inherited retinal disease (IRD). Targeted sequencing and WGS were found to have a similar sensitivity and specificity, but WGS identified an additional 14 clinically relevant variants. If applied to the whole cohort of 562, the authors hypothesized that WGS would provide an overall 29% (95% confidence interval, 15-45) uplift in diagnostic yield. They also noted, however, that creating a more targeted NGS panel would have a similar result.

Cirino et al. (2014) examined the validity of WGS in 41 patients with hypertrophic cardiomyopathy (HCM) who had undergone a HCM targeted next generation sequencing panel test. Twenty of the participants had pathogenic variants identified by targeted sequencing, and WGS detected 19 of them. Three additional variants were found in genes associated with HCM, but these genes are not typically included in HCM targeted sequencing panels. Additionally, 84 secondary (incidental) findings were uncovered. The authors concluded that WGS may provide advantages in being able to interrogate more genes, and give the opportunity for re-analysis over time, but noted that expertise in genomic interpretation is required to incorporate into care.

Dewey et al. (2014) conducted a pilot study to determine the resources required to identify and interpret clinically relevant genetic variation using WGS technologies and to evaluate clinical action prompted by WGS findings. An exploratory study of WGS was conducted in 12 adult participants at Stanford University Medical Center between November 2011 and March 2012. A multidisciplinary team reviewed all potentially reportable genetic findings. Five physicians proposed initial clinical follow-up based on the genetic findings. Depending on sequencing platform, 10% to 19% of inherited disease genes were not covered to accepted standards for single nucleotide variant discovery. Genotype concordance was high for previously described single nucleotide genetic variants (99%-100%) but low for small insertion/deletion variants (53%-59%). Curation of 90 to 127 genetic variants in each participant required a median of 54 minutes per genetic variant, resulted in moderate classification agreement between professionals, and reclassified 69% of genetic variants cataloged as disease causing in mutation databases to variants of uncertain or lesser significance. Two to 6 personal disease-risk findings were discovered in each participant, including 1 frameshift deletion in the BRCA1 gene implicated in hereditary breast and ovarian cancer. Physician review of sequencing findings prompted consideration of a median of 1 to 3 initial diagnostic tests and referrals per participant, with fair interrater agreement about the suitability of WGS findings for clinical follow-up. The authors concluded that in this exploratory study of 12 volunteer adults, the use of WGS was associated with incomplete coverage of inherited disease genes, low reproducibility of detection of genetic variation with the highest potential clinical effects, and uncertainty about clinically reportable findings.

Additional peer-reviewed literature on WGS consists primarily of case reports and small case series (Willig et al., 2015; Yuen et al., 2015; Jiang et al., 2013). The limited clinical experience with WGS causes gaps in interpreting variants of uncertain significance or other incidental findings. As a result, the benefits and risks of WGS testing are poorly defined and the role of WGS in the clinical setting has not yet been established.

WGS Cancer

Laskin et al. (2015) performed whole genome sequencing on the tumors of 100 individuals with incurable cancer, including 38 with refractory breast cancer, in the Personalized OncoGenomics (POG) study. Testing was completed in 78 patients. Of these, 55 patients received results that were considered "actionable" by a multi-disciplinary team. Twenty three patients received treatment that was driven by WGS results. Turnaround time was a challenge, and at the beginning of the study, results took >80 days to complete. By the end of the study, the results were completed in 50 days. The authors reported that there were limited treatment options available based on results, including even when considering available clinical trials.

Professional Societies

American College of Obstetricians and Gynecologists (ACOG)

In the Committee Opinion 682 (2016), ACOG states that “the routine use of whole-genome or whole-exome sequencing for prenatal diagnosis is not recommended outside of the context of clinical trials until sufficient peer-reviewed data and validation studies are published.”

American Academy of Neurology (AAN)/American Association of Neuromuscular and Electrodiagnostic Medicine (AANEM)

The AAN and AANEM have indicated that there is low level evidence to consider WES or WGS in selected individuals with congenital muscular dystrophy in who a genetic variation has not been identified through standard testing approaches. Individuals with congenital muscular dystrophy that do not have causative genetic variations identified through routine methods can be considered for WES or WGS when those technologies are clinically available. Evidence Level C (Kang et al., 2015).
American Society of Human Genetics (ASHG)
ASHG (Botkin et al., 2015) makes the following recommendations pertaining to the genetic testing of children and adolescents:

- Diagnostic testing:
  - Pharmacogenetic testing in children may be appropriate in the context of clear evidence of clinical utility.
  - Genetic testing should be limited to single gene or targeted gene panels based on the patient’s clinical presentation when appropriate. When WGS is performed, it is ethically acceptable to limit the analysis to a limited number of genes of interest.
  - Genome-scale sequencing is appropriate when prior, more limited genetic testing has failed to identify a causative variant. Genome-scale sequencing may be appropriate as an initial genetic test under certain circumstances.

American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP)
ACMG and AMP released guidance to laboratories in 2015 on how to evaluate variations found through next generation sequencing (NGS), including WES and WGS. They also highlighted the responsibility of the ordering provider in the process, stating “due to the complexity of genetic testing, optimal results are best realized when the referring healthcare provider and the clinical laboratory work collaboratively in the testing process”.

The guidelines highlight that healthcare providers need to be prepared to provide detailed information on other lab tests performed, clinical evaluations and testing, and patient phenotype. They need to understand that some results returned, such as “variants of unknown significance,” may not be actionable, or the clinical implication may be unknown for pathogenic mutations. Testing of additional family members may be required to interpret the test results of the patient. Finally, as new data emerges, the interpretation of a variant may change over time and the healthcare provider must be prepared to monitor and manage changing interpretations. As highlighted by ACMG and AMP, “variant analysis is at present imperfect and the variant category reported does not imply 100% certainty.”

American College of Medical Genetics and Genomics (ACMG)
In 2016, the ACMG released an updated policy statement on recommendations for reporting of secondary findings in clinical exome and genome sequencing. Four new genes were added to the list of recommended secondary findings, along with the elimination of one of the earlier genes from the 2013 list. The new, updated secondary findings list includes 59 medically actionable genes recommended for return in clinical genomic sequencing (Kalia et al., 2016).

The ACMG Board of Directors (2012) published a policy statement regarding use of genomic testing that recommends that WGS/WES should be considered in the clinical diagnostic assessment of a phenotypically affected individual when:

- The phenotype or family history data strongly implicate a genetic etiology, but the phenotype does not correspond with a specific disorder for which a genetic test targeting a specific gene is available on a clinical basis.
- A patient presents with a defined genetic disorder that demonstrates a high degree of genetic heterogeneity, making WES or WGS analysis of multiple genes simultaneously a more practical approach.
  - A patient presents with a likely genetic disorder, but specific genetic tests available for that phenotype have failed to arrive at a diagnosis.
  - A fetus with a likely genetic disorder in which specific genetic tests, including targeted sequencing tests, available for that phenotype have failed to arrive at a diagnosis.
  - WGS/WES should not be used at this time as an approach to prenatal screening.
  - WGS/WES should not be used as a first-tier approach for newborn screening.

U.S. FOOD AND DRUG ADMINISTRATION (FDA)

No FDA-approved tests for WES or WGS are available at this time.

REFERENCES
The foregoing Oxford policy has been adapted from an existing UnitedHealthcare national policy that was researched, developed and approved by UnitedHealthcare Medical Technology Assessment Committee. [2018T0589B]

American College of Medical Genetics and Genomics (ACMG) Board of Directors. Policy statement points to consider in the clinical application of genomic sequencing. May 2012. Available at:


Whole Exome and Whole Genome Sequencing
UnitedHealthcare Oxford Clinical Policy

POLICY HISTORY/REVISION INFORMATION

<table>
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| 03/01/2018 | • Updated list of related policies; added reference links to policies titled: o Chromosome Microarray Testing  
• Revised coverage rationale:  
  o Modified language pertaining to Whole Exome Sequencing to indicate:  
    ▪ Whole Exome Sequencing (WES) is proven and/or medically necessary for diagnosing or evaluating a genetic disorder when the results are expected to directly influence medical management and clinical outcomes and all of the following criteria are met:  
      - Clinical presentation is nonspecific and does not fit a well-defined syndrome for which a specific or targeted gene test is available; if a specific genetic syndrome is suspected, a single gene or targeted gene panel should be performed prior to determining if WES is necessary; and  
      - WES is ordered by a board-certified medical geneticist, neonatologist, neurologist, or developmental and behavioral pediatrician; and  
      - One of the following:  
        ▪ The clinical presentation or clinical and family history strongly suggest a genetic cause for which a specific clinical diagnosis cannot be made with any clinically available targeted genetic tests; or  
        ▪ There is a clinical diagnosis of a genetic condition where there is significant genetic heterogeneity and WES is a more practical approach to identifying the underlying genetic cause than are individual tests of multiple genes; or  
        ▪ There is likely a genetic disorder and multiple targeted gene tests that have failed to identify the underlying cause  
    ▪ WES is unproven and/or not medically necessary for all other indications, including but not limited to the following:  
      - Screening and evaluating disorders in individuals when the above criteria are not met  
      - Prenatal genetic diagnosis or screening  
      - Evaluation of fetal demise  
      - Preimplantation genetic diagnosis or screening in embryos  
      - Molecular profiling of tumors for the diagnosis, prognosis or management of cancer  
  o Replaced language indicating:  
    ▪ "Comparator (e.g., parents or siblings) WES is proven and medically necessary for evaluating a genetic disorder when the above criteria have been met and WES is performed concurrently or has been previously performed on the patient" with "comparator (e.g., parents or siblings) WES is proven and/or medically necessary for evaluating a genetic disorder when the above criteria have been met and WES is performed concurrently or has been previously performed on the patient"
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<td>disorder when the above criteria have been met and WES is performed concurrently or has been previously performed on the individual”</td>
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<td>“Whole Genome Sequencing (WGS) is unproven and not medically necessary for screening and evaluating any genetic disorder” with “Whole Genome Sequencing (WGS) is unproven and/or not medically necessary for screening and evaluating any genetic disorder”</td>
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<td>Modified language pertaining to clinical evidence/study findings for Whole Genome Sequencing to indicate:</td>
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<td>Although WGS has the potential to identify causal variants for a wide variety of conditions that may be missed with other technologies, as well as to identify predictive biomarkers, the information derived from WGS has not yet been translated into improved outcomes and changed medical management</td>
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<td>Updated supporting information to reflect the most current description of services, clinical evidence, FDA information, and references</td>
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